REMARKS/ARGUMENTS

Reconsideration of the above-identified application is respectfully requested in view of the foregoing amendment and the following remarks. As a result of an earlier restriction requirement, Claims 1-26 and 39-40 are cancelled, and Claim 38 is withdrawn. In view of the examiner's earlier restriction requirement, Applicants retain the right to present claims 1-26, 38, and 39-40 in divisional applications. Claim 27 has been amended in accordance with Examiner Robinson's remarks, and Claims 27-37 remain in the case.

Thermal hysteresis proteins and their nucleotide sequences, which are derived from the Tenebrionoidea Superfamily, lower the freezing point of a solution without affecting the melting point. This thermal hysteretic behavior of antifreeze proteins is attributed to a specific protein-ice interaction that restricts ice growth, but not ice melt, hence creating a difference between the freezing and melting point of a solution.

The present invention provides nucleic acid sequences encoding proteins having antifreeze properties and compositional characteristics of an insect Type III AFP, wherein the nucleic acid sequences are derived from the Genus Tenebrio, including the species *Tenebrio molitor* (Tm), the yellow mealworm beetle. These insect Type III antifreeze proteins ("AFPs") display more potent thermal hysteresis activity than that seen with fish AFPs and AFGP (antifreeze glycoproein), and are further subject to enhancement by activating substances, also a component of the present invention.

The present invention also provides for the incorporation of the Tm AFP into cells and other media. The freezing point depression activity of the Tm 12.86 family of peptides, their capabilities of masking potential ice nucleators, ability to stabilize supercooled states, and prevent ice recrystallization, coupled with the ability to clone and express these genes in large amounts of recombinant protein make their applicability and availability for commercial use

ideal. The *T. molitor* AFP has potentially diverse applications, including but not limited to, the food industry, cryopreservation, and to the making of humans and the cells of animals more resistant to cold temperatures.

Claim 37 was rejected under 35 U.S.C. §112, first paragraph, because in the opinion of Examiner Robinson, the specification, while being enabling for a method for providing antifreeze or recrystallization inhibition properties to a subject formulation, may not reasonably provide enablement for said method wherein the polynucleotides for the activated protein are used to create transgenic or gene-modified plants, crops, fish, or animals having greater tolerance to cold climatization. However, Applicants believe that advances in gene therapy technology have proven therapeutic benefits not only in plants, crops, fish, and animals, but also in humans. People skilled in the art of gene transfer technology are capable of making transgenic cells.

In an article published in 2000, Gene Therapy of Human Severe Combined Immunodeficiency (SCID) -X1 Disease (Cavazzanna-Calvo, M. et al. [2000] Science 288:669-672), correction of SCID-X1 patients (up to 10 months) of the SCID-X1 phenotype was achieved in two patients using gene transfer technology. SCID is caused by the absence of γc cytokine receptor deficiency that leads to an early block in T and NK lymphocyte differentiation. Using a retro-viral vector, γc cytokine gene was successfully infused into cells ex vivo of two patients (both ADA-deficient, and 11 and 8 months old respectively). The amount of T-lymphocyte counts increased from day 30 in Patient 1, whereas γc-expressing T-cell counts became detectible in the blood of Patient 2 at day 60. Subsequently T-cell counts increased after 8 months. This methodology has resulted in the sustained correction (up to 10 months) of the SCID-X1 phenotype in both of two patients.

It is therefore possible for one skilled in the art to create transgenic human cells without undue practice and experimentation. The above is a working example. No large quantity of experimentation is necessary to transduce an antifreeze gene into plants, crops, fish, animals, and even

human cells using a cloning vector, as Applicants suggest, since it is done similarly to the way the adenosine-deaminase production gene was incorporated into human cells in the aforementioned trial using a retro-viral vector. Incorporation of the antifreeze gene into any cell should make the cell more tolerant of lower temperatures, causing it to be more resistant to recrystallization.

Predictability of the success of creating a whole organism/animal such as a human being that has greater tolerance for cold climates is supported by the Cavazzanna-Calvo article. In contrast, the article written by Eliot (Eliot, Science [1995] 269:1050-1055), cited by Examiner Robinson, may have described the state of the art in 1995 when it was published, but it was not the state of the art in 2001, the year this patent application was submitted. The therapeutic benefits to humans of gene transfer technology was illustrated by the Cavazzanna-Calvo article showing how a reversal of a SCID-X1 deficiency was sustained in both of two patients using gene transfer technology. Similarly here, by transferring the antifreeze gene into human cells using the method duly described in the patent application, which is similar to that used in the Cavazzanna-Calvo article, humans will be more resistant to lower temperatures. Therefore, the ". . . scope of claims [do] bear [at least] a reasonable correlation to scope of enablement provided by the specification to persons of ordinary skill in the art . . ." (*In Re Fisher*, 427 F.2d 833, 839, 166 USPQ 18, 24 (CPPA 1970)).

Claims 27-37 were rejected under 35 U.S.C. §112, second paragraph, as failing to set forth the subject matter of the Applicants' invention. Claim 27 has been amended in accordance with the Examiner's remarks. No new matter has been added. As a result, the rejection under 35 U.S.C. §112, of claim 27, and dependent claims 28-37 has been traversed.

Claims 27-36 were rejected under 35 U.S.C. §102(b), as being anticipated by RUBINSKY et al. (U.S. Patent No. 5,358,931, October 25, 1994). However, the present invention differs significantly from, and is in no way anticipated,

suggested, or motivated by the disclosure of RUBINSKY et al. The disclosure of RUBINSKY et al. claims a method for incorporating the antifreeze protein isolated from fish ("fish AFP") into cells and other media. The present invention provides for a method of incorporating the *Tenebrio molitor* type III antifreeze protein ("TmAFP") into cells and other media. For the following reasons, TmAFP is different from fish AFP, which makes the disclosure of RUBINSKY et al. significantly different from the present invention.

TmAFP is 8.4 kDa in size (Liou et al. [1999] Biochemistry 38:11415-11424). The dominant structural element of all TmAFP isoforms is a tandemly-linked, 12 amino acid repeat, with the consensus sequence CTxSxxCxxAxT (Liou et al. [1999] Biochemistry 38:11415-11424). Each repeat contains two cysteines spaced six residues apart. Thus cysteine is repeated at six-residue intervals throughout most of the protein, and almost every second cysteine is flanked by two threonines (Liou et al. [1999] Biochemistry 38:11415-11424). This highly conserved TCT sequence is thought to play a major role in TmAFP's binding to ice (Jia, Zongchao and P. Davies [2002] Trends in Biochemical Sciences 27(2):101-106).

The crystal structure of TmAFP is a right-handed β-helix (Jia, Zongchao and P. Davies, Trends in Biochemical Sciences [2002] 27(2):101-106) with a rung of disulfide bridges down the middle (Duman, J. G. [2001] Annu. Rev. Physiol. 63:327-357; Liou et al. [1999] Biochemistry 38:11415-11424). Each internally disulfide-bonded 12-amino acid repeat is stacked side-by-side to form the β-helix (Liou et al. [1999] Biochemistry 38:11415-11424). The β-helix coil presents a rigid array of TCT residues on one side of the protein that, along with bound water molecules, is able to mimic the ice lattice of the prism and basal planes of the ice, and is thus able to provide more effective coverage of the ice surface compared to the fish AFPs (Graether, S. P. and B. D. Sykes [2004] Eur. J. Biochem. 271:3285-3296]; Liou et al. [1999] Biochemistry 38:11415-11424); Duman, J. G. [2001] Annu. Rev. Physiol. 64:327-357).

RUBINSKY et al. claim a method for preservation of mammalian cells using a liquid containing the "thermal hysteresis proteins isolated and purified from a polar fish species" (U.S. Patent No. 5,358,931, October 25, 1994). The claims of RUBINSKY et al. are limited to polar fish AFP. Fish AFP is different from TmAFP both in structure and in activity.

No fish AFP is the same or similar to TmAFP in size, structure, or amino acid repeat. Fish AFP Type I is an alanine rich α-helix. It is 3-5kdA in size and contains an 11 amino acid repeat. Type II fish AFP is 14-24kDa in size and is a C-type lectin fold of mixed alpha, beta, and loop structure. Type III fish AFP is 7kDa in size and is a globular protein containing short beta strands. Neither fish AFP Type II nor fish AFP Type III contains a repeating amino acid sequence or polar groups that can bind ice. (Fletcher et al. [2001] Annu. Rev. Physiol. 63:359-390)

Type	Classification	Size (kDa)	Repeat
TmAFP	Right-handed beta-helix	8.4	12 amino acid repeat with a highly conserved TCT sequence.
Fish Type I	Alanine rich alpha-helix	3-5	11 amino acid repeats
Fish AFP Type II	C-type lectin fold of mixed alpha, beta, and loop structure	14-24	none
Fish AFP Type III	Globular protein containing short beta-strands	7	none

(Jia, Zongchao, and P. Davies [2002] Trends in Biochem. Sci. 27(2):101-106; Liou et al. [1999] Biochemistry 38:11415-11424; Davies, P. L. and C. L. Hew [1990] The FASEB Journal 4: 2460-2468; Graether, S. P. and B. D. Sykes [2004] Eur. J. Biochem. 271:3285-3296; Fletcher et al. [2001] Annu. Rev. Physiol. 63:359-390)

Insect AFPs must protect against freezing temperatures that are considerably colder than that necessary for fish survival. Polar fish survive in seawater at -1.9°C (Jia, Zongchao, and P. Davies [2002] Trends in Biochem. Sci. 27(2):101-106; Fletcher at al [2001] Annu. Rev. Physiol. 63:359-390), whereas insects in polar regions survive temperatures of -50 to -70°C or lower (Duman, J. G. [2001] Annu. Rev. Physiol. 63:327-357). The hyperactivity of the insect AFP results in 10-100X greater freezing point depression activity on a molar basis than that produced by fish antifreeze proteins (Graether, S. P. and B. D. Sykes [2004] Eur. J. Biochem. 271: 3285-3296; Liou et al. [1999] Biochemistry 38:11415-11424).

Due to its hyperactivity, TmAFP will protect against recrystallization better than fish AFP Types I, II, and III, by providing a more depressed non-colligative freezing point than fish AFP will. The Tm AFP will provide better recrystallization inhibition than the fish AFP when incorporated into the following media including but not limited to:

(1) Plant, produce or fish cells in an amount sufficient to provide antifreeze protection;

(2) A region of a target tissue in an amount sufficient to provide antifreeze protein controlled limited tumor cell or target tissue cryoinjury during cryosurgery;

(3) Hypothermic solutions or bathing media to reduce cold damage in order to provide cryogenic or hypothermic preservation of cells and tissues by incorporating said protein into said cells, tissue, or cell membranes in a controlled amount sufficient to provide antifreeze protection;

(4) De-icing formulations or used on surfaces to reduce existing ice buildup or abate the formation of ice buildup on surfaces of road, aircraft, household products, cosmetic products, machinery and plants; and

(5) A food product in an amount sufficient to provide antifreeze protection to improve the quality of food by abating freezing of solutions, freezer burn, or degradation due to cold storage.

TmAFP will also provide better recrystallization inhibition than fish AFP when the polynucleotides for the activated protein are used to create transgenic or gene-modified plants, crops, fish, or animals having greater tolerance to cold climatization.

The disclosure of RUBINSKY et al. is limited to the incorporation of fish AFP, whereas the present invention provides for incorporation of Tenebrio Type III AFP. The disclosure of RUBINSKY et al. in no way anticipates, suggests, or motivates Applicants' invention. TmAFP is different both in structure and activity from fish AFP. Therefore, the present invention is not anticipated by the prior disclosure by RUBINSKY et al., and Applicants respectfully traverse the rejection of claims 27-36 under 35 U.S.C. §102(b), as being anticipated by RUBINSKY et al.

Applicants believe that claims 27 - 37 are allowable and therefore respectfully request that claims 27 - 37 be allowed and the application be passed to issue.

Respectfully submitted,
MARK LEVY & ASSOCIATES, PLLC

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to:

Mail Stop _____
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

On July 18, 2005
(Date of Deposit)

Edward Vytlacil 7/18/2005
Edward Vytlacil, Reg. No. 53,729 (Date)
Attorney

By: Edward Vytlacil
Registration No. 53,729
Attorney for Applicants
Press Building - Suite 902
19 Chenango Street
Binghamton, New York 13901
Phone: (607) 722-6600

Amendments to the Drawings:

The attached sheet of drawings includes changes to Fig. 2.6c. This sheet which includes Fig. 2.6c replaces the original sheet including Fig. 2.6c. This figure now shows proper spelling of "residue" in the heading beginning with "A".

Attachment: Replacement Sheet
Annotated Sheet Showing Changes

A. Mature Tm 13.17 amino acid residue

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  1  LTEAQIEKLN KISKKCQNES GVSQEIITKA RNGDWEDDPK LKRQVFCVAR

 51  NAGLATESGE VVVDVLREKV RKVTDNDEET EKIINKCAVK RDTVEETVFN

101  TFKCVMKNKP KFSPVD
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B. Summary of the composition analysis for the mature Tm 13.17 sequence:

Residue	Number	Mole Percent
A = Ala	6	5.172
B = Asx	0	0.000
C = Cys	4	3.448
D = Asp	8	6.897
E = Glu	**13**	**11.207**
F = Phe	4	3.448
G = Gly	4	3.448
H = His	0	0.000
I = Ile	6	5.172
K =Lys	**16**	**13.793**
L = Leu	5	4.310
M = Met	1	0.862
N = Asn	8	6.897
P = Pro	3	2.586
Q = Gln	4	3.448
R = Arg	6	5.172
S = Ser	5	4.310
T = Thr	8	6.897
V =Val	**14**	**12.069**
W = Trp	1	0.862
Y = Tyr	0	0.000
Z = Glx	0	0.000

Molecular weight = 13171.96; Residues = 116; Average Residue Weight = 113.551

Charge = 1; Isoelectric point = 7.74.

FIG 2.6c